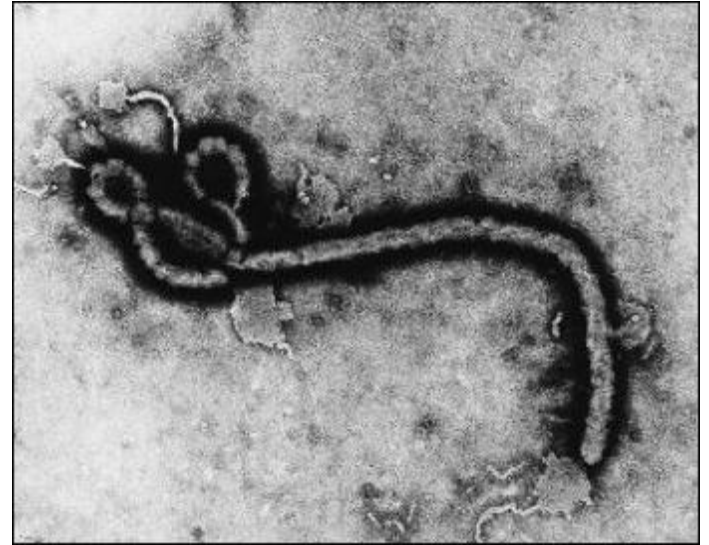


*Ebola: A Terrifying Challenge?*

or

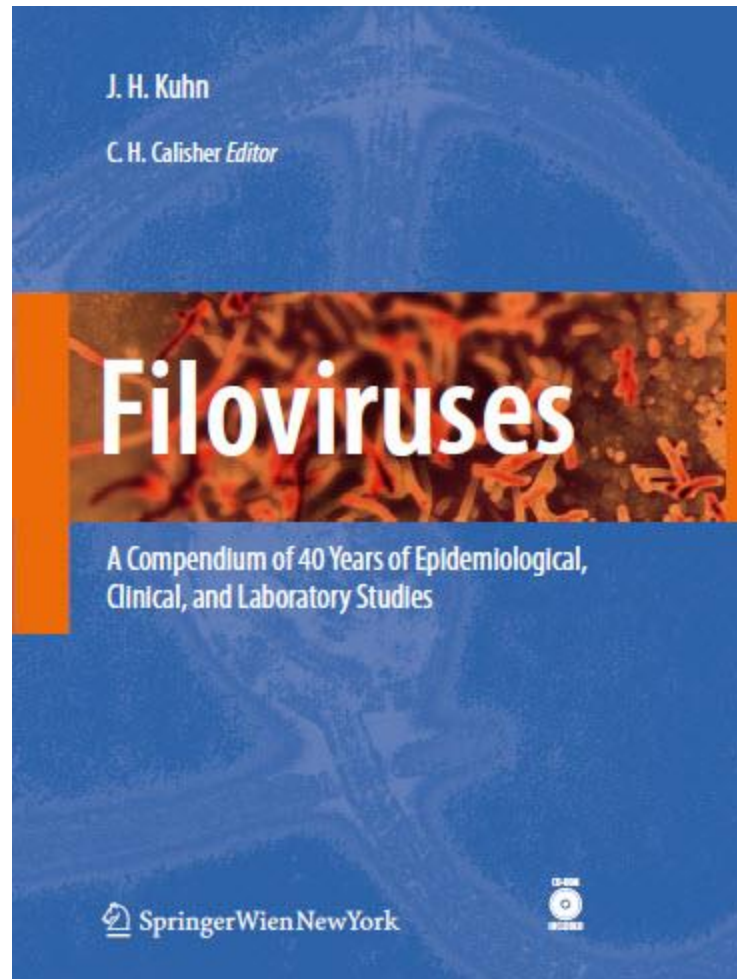
*Demystifying Filoviruses!*



**Presented on January 27, 2015, by Jens H. Kuhn, MD, PhD, PhD, MS**

**Virology Lead, NIH/NIAID Integrated Research Facility at Fort Detrick (IRF-Frederick), Frederick,  
MD**

# Filoviruses



# Filoviruses

## The Soviet Biological Weapons Program

### A History

Milton Leitenberg

Raymond A. Zilinskas

With Jens H. Kuhn

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## THE SOVIET BIOLOGICAL WEAPONS PROGRAM



A HISTORY

MILTON LEITENBERG  
RAYMOND A. ZILINSKAS

# Definition of Viral Hemorrhagic Fevers



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## Definition of Viral Hemorrhagic Fevers

- Medical category for severe clinical syndromes
  - Known for centuries
  - Vastly different case-fatality rates
  - Caused by unrelated agents
  - Transmitted by different means
  - Difficult differential diagnosis
-

# Characteristics of Human Viral Hemorrhagic Fever Agents

- VHF agents are usually highly infectious, but not very contagious
- Initial infection with VHF agents occurs either directly via hematophagous arthropods (arthropod-borne viruses, arboviruses) or indirectly via the inhalation of dried rodent excreta or secretions (rodent-borne viruses, roboviruses); further transmission usually requires direct person-to-person contact
- Human VHF agents can be assigned to four distinct viral families

# Human Viral Hemorrhagic Fever Agents

- *Arenaviridae*
- *Bunyaviridae*
- *Filoviridae*
- *Flaviviridae*

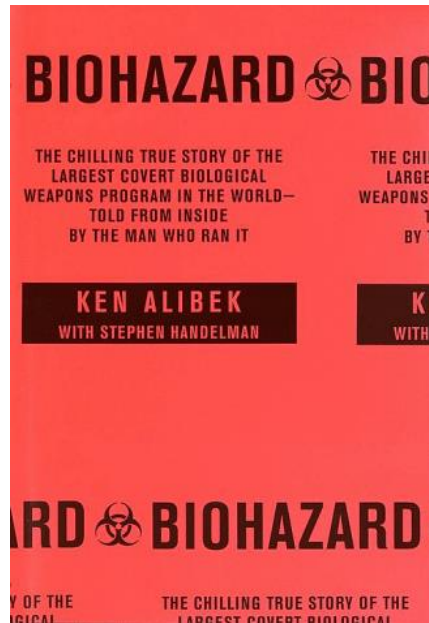
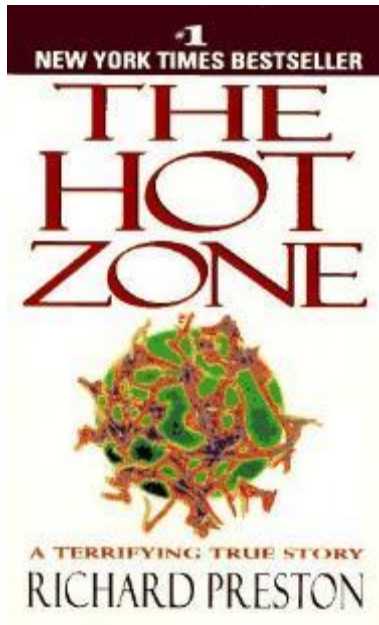
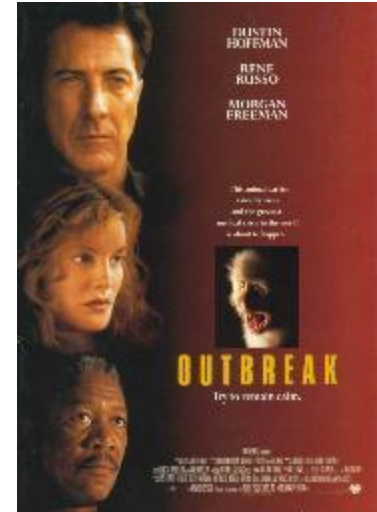


# The Cultural Model of “Ebola” in North America and Europe

space suits

immigration

virus hunters



terrorists



rain forests



# Filoviruses - Overview

- Host are unclear
- Etiologic agents of rare severe hemorrhagic fever epidemics (EVD, MVD)
- May be etiologic agents of hemorrhagic fever-like epizootics
- Researched in the former Soviet BW program
- No specific prophylaxis
- No specific treatment
- Hence classified as Class/Risk Group 4 Pathogens, NIAID Category A Priority Pathogens, and Tier 1 Select Agents in the US

# Facts about Ebola

## What is Ebola?

- . A severe disease in humans and animals which spreads fast

## How does Ebola spread?

- . It can be spread easily and kill in a short time
- . Ebola can even spread from dead bodies of people or animals that were infected.

- . You have to have direct contact with the blood, saliva, urine, stool, sweat, semen of an infected person or infected animal.
- . People who are most at risk are health workers, close family members and friends of the infected person. Also people who eat bush meat or in contact with wild animals (monkeys, bats, and baboon), can also get Ebola.
- . Incubation period—How long does it take for the virus to be seen in a person?  
2—21 Days

## What are the **signs** and **symptoms** of Ebola Virus Disease?



### **Early signs and symptoms:**

- . Fever
- . Severe headache
- . Joint and muscle pain
- . Sore throat
- . Chills (feeling cold)

### **Late signs and symptoms :**

- . Sickness
- . vomiting
- . Diarrhea (may be bloody)
- . Red eyes running water
- . Rash (red bumps on the body)
- . Chest pain and cough
- . Stomach pain
- . Severe weight loss
- . Hiccups
- . Bleeding from the nose, mouth, rectum, eyes and ears



**Abortion (miscarriage) and heavy vaginal bleeding are other signs in pregnant women**





**DO**



**Always wash  
your hands with  
soap and**



**Always cook your  
food properly**



**Go to health facility  
anytime you have  
head ache, fever,  
pain,  
diarrhea, red  
eyes rash and  
vomiting**



**Tell everyone you  
meet about  
Ebola so they can  
be informed**



**Call for help or  
questions**

0886520581 or 0886374733



**DO NOT**



**Do not touch people  
with signs of Ebola or  
have died of Ebola**



**Do not touch clothes &  
bed cloths of people  
who have died of Ebola**



**Do not touch vomit,  
saliva, urine, blood and poo  
of people who have signs  
and symptoms of Ebola**



**Do not play with  
monkeys and  
baboons**



**Do not eat bush  
meat**



**Do not eat plums  
eaten by bats**



# Filovirus Taxonomy

Year	ICTV Taxonomy
2011-present	<p>Order <i>Mononegavirales</i></p> <p>Family <i>Filoviridae</i></p> <p>Genus <i>Marburgvirus</i></p> <p>Species <i>Marburg marburgvirus</i></p> <p>Virus: Marburg virus (MARV)</p> <p>Virus: Ravn virus (RAVV)</p> <p>Genus <i>Ebolavirus</i></p> <p>Species <i>Bundibugyo ebolavirus</i></p> <p>Virus: Bundibugyo virus (BDBV)</p> <p>Species <i>Taï Forest ebolavirus</i></p> <p>Virus: Taï Forest virus (TAFV)</p> <p>Species <i>Reston ebolavirus</i></p> <p>Virus: Reston virus (RESTV)</p> <p>Species <i>Sudan ebolavirus</i></p> <p>Virus: Sudan virus (SUDV)</p> <p>Species <i>Zaire ebolavirus</i></p> <p>Virus: Ebola virus (EBOV)</p> <p>Genus <i>Cuevavirus</i></p> <p>Species <i>Lloviu cuevavirus</i></p> <p>Virus: Lloviu virus (LLOV)</p>



# Filovirus Taxonomy Should Be Understood

Order Carnivora (all carnivores)  
Family Canidae (all dogs)  
Genus *Vulpes* (all true foxes)  
Species *Vulpes vulpes* (all red foxes)

Red fox  
Pack 1  
Individual 1, 2, 3

Order *Mononegavirales* (all mononegaviruses)  
Family *Filoviridae* (all filoviruses)  
Genus *Marburgvirus* (all marburgviruses)  
Species *Marburg marburgvirus* (all Marburg marburgviruses)

Marburg virus  
Variant 1  
Isolate 1, 2, 3

		In terms of cars...
Order	<i>Mononegavirales</i>	Motor vehicles
Family	<i>Filoviridae</i>	Car
Genus	<i>Ebolavirus</i>	Mitsubishi
Species	<i>Zaire ebolavirus</i>	Mitubishi Sigma
	<i>Tai Forest ebolavirus</i>	Mitsubishi Lancer
	<i>Sudan ebolavirus</i>	Mitsubishi Eclipse
	<i>Bundibugyo ebolavirus</i>	Mitsubishi Mirage
	<i>Reston ebolavirus</i>	
Variant	Ebola virus H.sapiens-tc/COD/1995/Kikwit-9510621	Mitsubishi Sigma, 1989, silver, ABC 321

# Application to 2014



Full name: Ebola virus/H.sapiens-<suffix>/<country>/2014/Makona-<isolate designation>

Shortened name: EBOV/H.sap/<country>/14/Mak-<isolate designation>

Abbreviated name: EBOV/Mak-<isolate designation>

Full name: Ebola virus/H.sapiens-<suffix>/<COD>/2014/Lomela-<isolate designation>

Shortened name: EBOV/H.sap/<COD>/14/Lom-<isolate designation>

Abbreviated name: EBOV/Lom-<isolate designation>

# Marburgvirus Epidemiology



Grivet  
(*Chlorocebus aethiops*)





# Marburgvirus Epidemiology



# Ebola virus Epidemiology

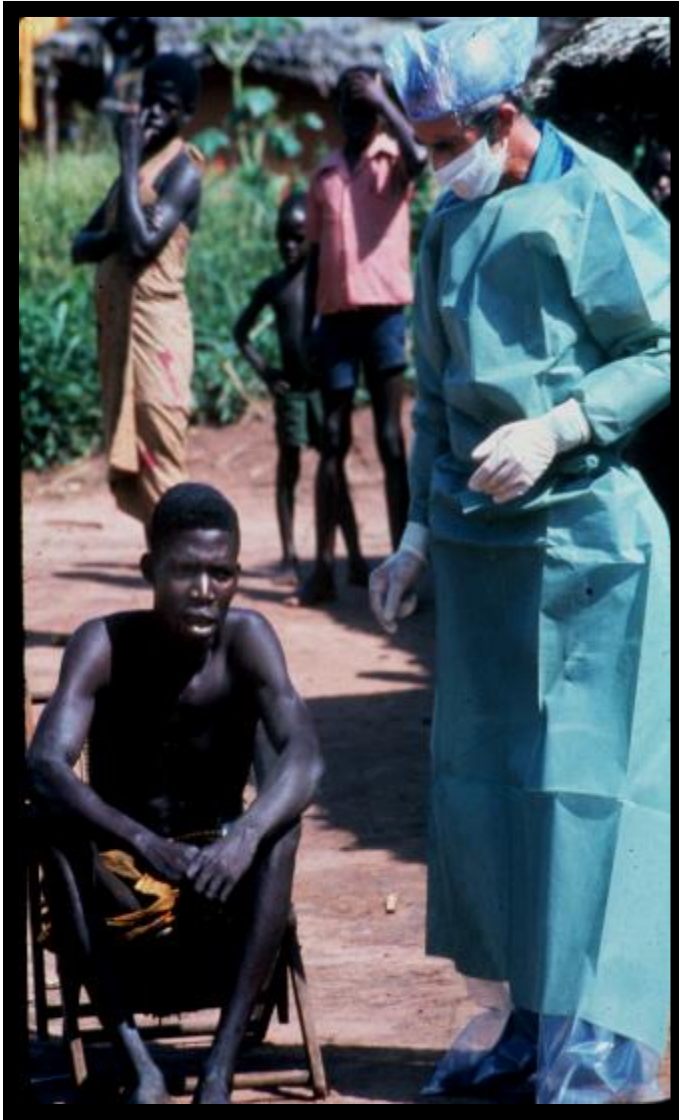
Month	Day	Event
August 1976	26	First patient presents with fever in Yambuku; receives chloroquine injection, fever resolves in four days
September 1976	1 16 23-25 30	Zaire Ebola hemorrhagic fever begins in first patient Local clinician reviews 17 patients; reports unknown disease to Kinshasa First medical team visit from Kinshasa; typhoid fever suspected; vaccination; evacuation of a Belgian nun to capital Hospital in Yambuku closed; 11 of 17 staff members dead
October 1976	2-6 3 8-12 13-14 14 18 19-27 30	Second medical team visit from Kinshasa; collection of specimens Health zone quarantined by minister Transmission occurring in Kinshasa hospital Filovirus identified by electron microscopy in Belgium, UK, and USA New virus ( 'Ebola' ) identified in USA International commission formed Survey team to Yambuku; reports active cases in eight villages Airlift of surveillance teams to northeastern Zaire and to the border with neighboring Sudan
November 1976	2 5 16	Plasmapheresis program begins with convalescent patients Last case dies Surveillance, research and clinical care support arrives in Yambuku
December 1976	16	Emergency officially over
January 1977	28	Plasmapheresis program ends

# Ebola virus Epidemiology





# Ebola virus Epidemiology



# Ebola virus Epidemiology

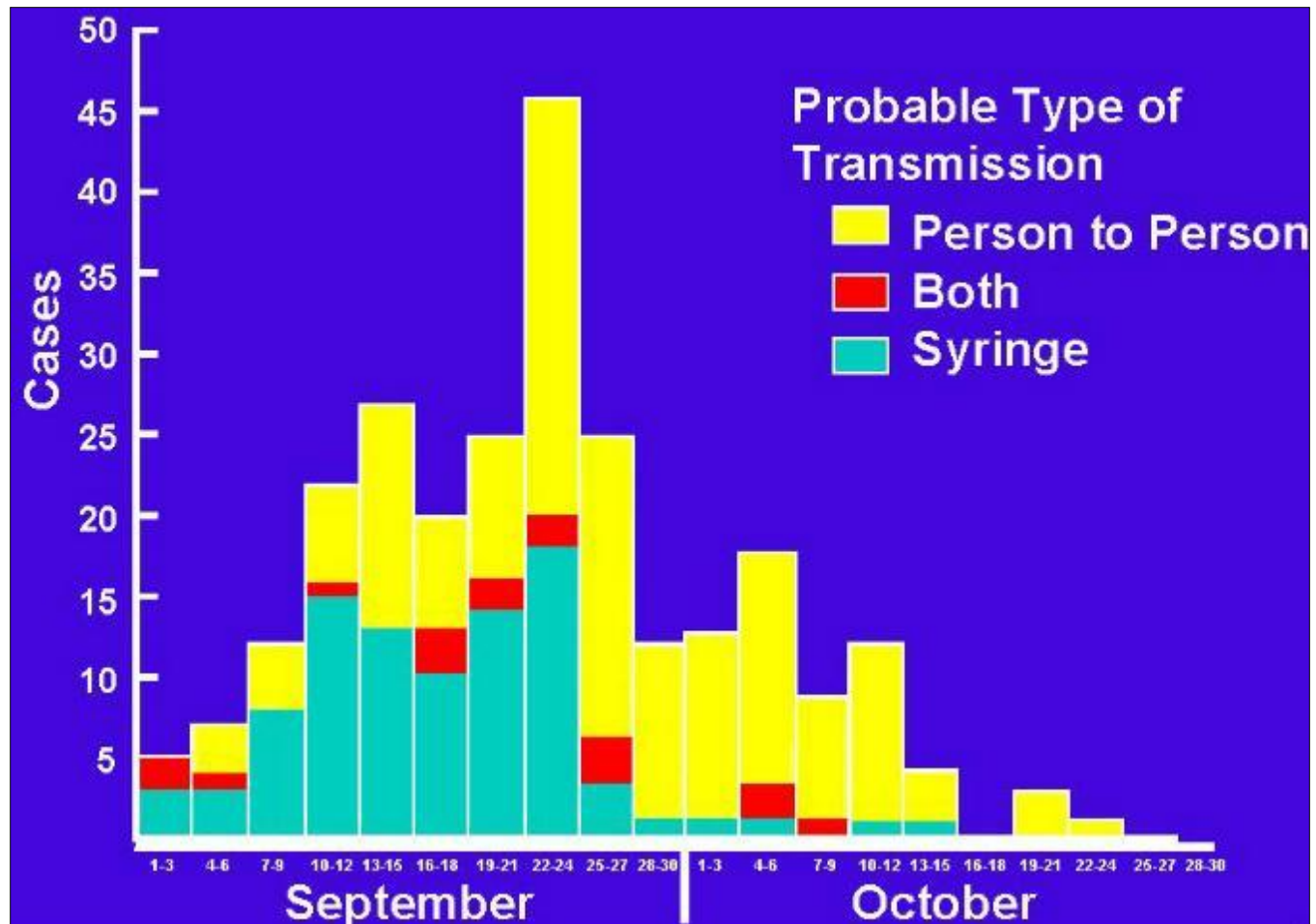


# Ebola virus Epidemiology





# Ebola virus Epidemiology



# Ebola virus Epidemiology



# Ebola virus Epidemiology



Bush pig/red river hog (*Potamochoerus porcus*)



Blue duiker (*Cephalophus monticola*)



Black-backed duiker (*Cephalophus dorsalis*)



Central chimpanzee (*Pan troglodytes troglodytes*)



Western lowland gorilla (*Gorilla gorilla gorilla*)



# Sudan virus Epidemiology



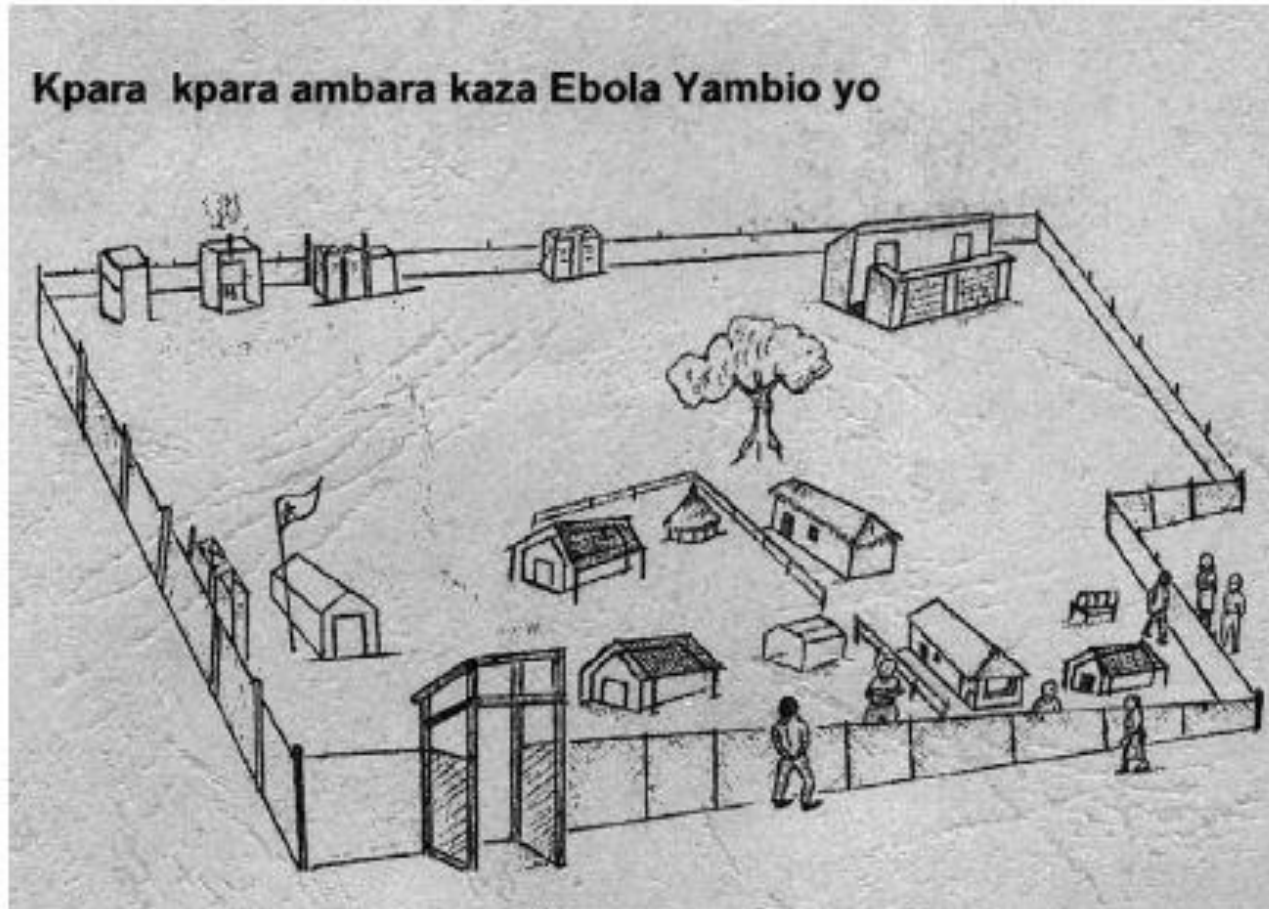
Olive baboon (*Papio anubis*)

# Sudan virus Epidemiology





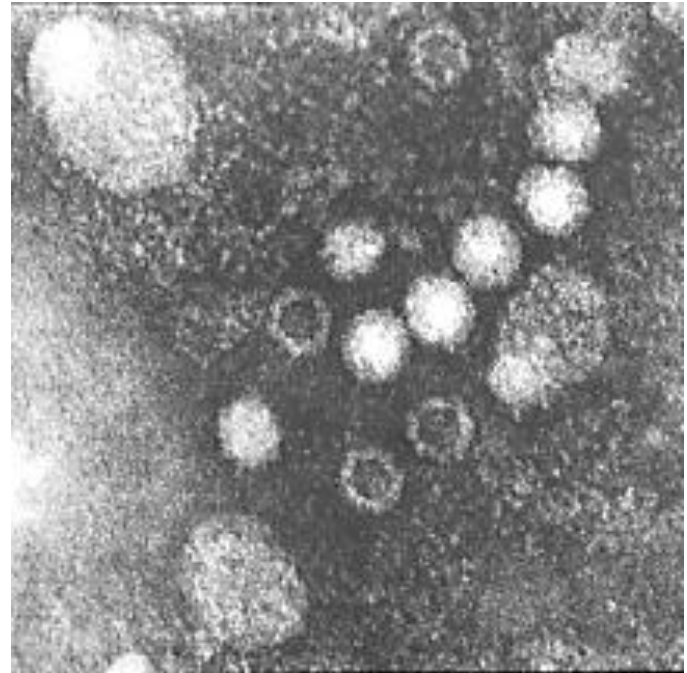
# Sudan virus Epidemiology



# Reston Virus Epidemiology

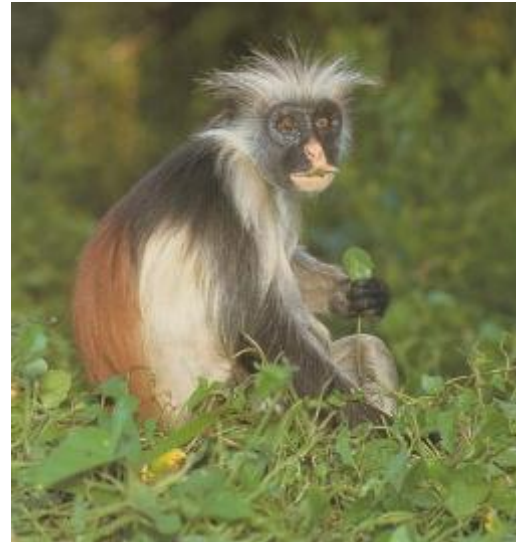


Crab-eating macaque (*Macaca fascicularis*)



Simian hemorrhagic fever virus (species *Simian hemorrhagic fever virus*, genus *Arterivirus*, family *Arteriviridae*)

# Tai Forest Virus Epidemiology



# Filovirus Transmission

- Burial ceremonies play a significant role in the transmission
- Filoviruses are transmitted by direct contact with blood, bodily secretions or tissues of infected animals or humans
  - Viral titers often approach  $10^7$  pfu/ml
  - The  $LD_{50}$  for rodents and nonhuman primates is thought to be 1-10 pfu
  - 1  $\mu$ l of blood (the equivalent of a needle stick) is thought to contain  $\approx 1,000$   $LD_{50}$
- Health-care workers often become infected while treating patients through close contact without the use of correct infection control precautions and adequate barrier-nursing procedures
- Although highly infectious, filoviruses are not very contagious
- Although animals are readily infected by the aerosol route, aerosol transmission has never been observed during natural outbreaks



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# Clinical Presentation

- Filovirus infections are characterized by the sudden onset of fever, intense weakness, myalgia, headache, and sore throat. This is often followed by vomiting, diarrhea, morbilliform rash, impaired kidney and liver function, and in some cases, hemorrhages. Laboratory findings show low counts of white blood cells and platelets as well as elevated liver enzymes and elevated D-dimers
  - Incubation period is 2 – 21 days
  - Currently limited supportive care is the only available treatment option
-

# Marburg virus Disease (DRC 1998-2000)

Clinical symptom	Frequency observed in 22 survivors (%)	Frequency observed in 107 fatal cases (%)
Abdominal pain	59	57
Anorexia	77	72
Arthralgia or myalgia	55	55
Bleeding from puncture sites	0	7
Bleeding from the gums	23	36
Bleeding from any site	59	71
Chest pain	18	4
<b>Conjunctival injection</b>	<b>14</b>	<b>42</b>
Cough	9	5
Diarrhea	59	56
Difficulty breathing	36	58
<b>Epistaxis</b>	<b>18</b>	<b>34</b>
Fever	100	92
Headaches	73	79
Hematemesis	68	76
Hematoma	0	3
Hemoptysis	9	4
<b>Hiccups</b>	<b>18</b>	<b>44</b>
Lumbar pain	5	8
Malaise or fatigue	86	83
Melena	41	58
Nausea and vomiting	77	76
Petechiae	9	7
Sore throat, odynophagia, or dysphagia	43	43

# Marburg virus Disease (Rhodesia 1975)



# Ebola Virus Disease (Zaire, 1995)

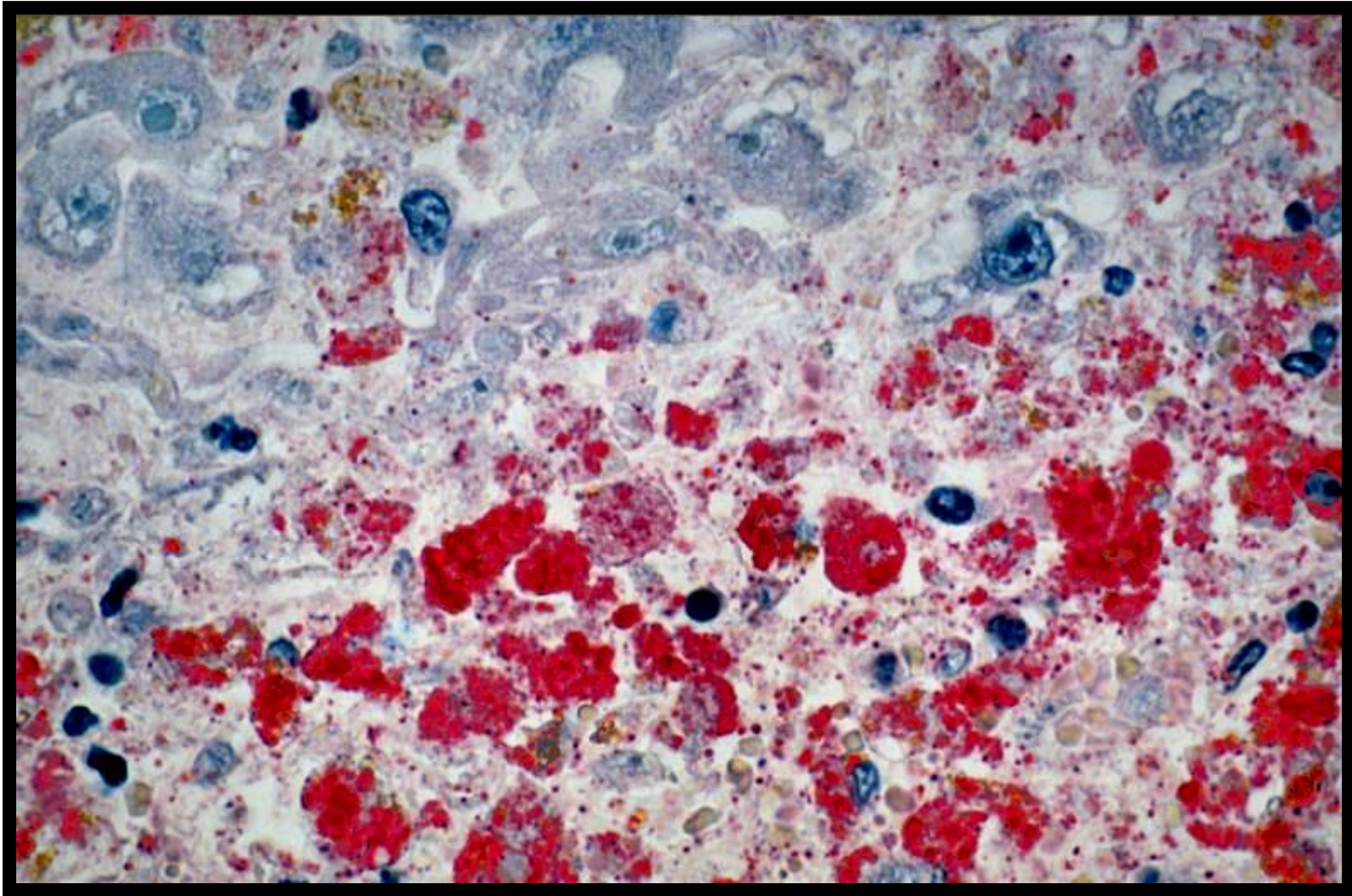
Clinical symptom	Frequency observed in 19 survivors (%)	Frequency observed in 84 fatal cases (%)
Abdominal pain	68	62
Abortion	5	2
Anorexia	47	43
Anuria	0	7
Arthralgia or myalgia	79	50
Asthenia	95	85
Bleeding from puncture sites	5	8
<b>Bleeding from the gums</b>	<b>0</b>	<b>15</b>
Bloody stools	5	7
Chest pain	5	10
Conjunctival injection	47	42
Convulsions	0	2
<b>Cough</b>	<b>26</b>	<b>7</b>
Diarrhea	84	86
Dysesthesia	5	0
Epistaxis	0	2
Fever	95	93
<b>Headaches</b>	<b>74</b>	<b>52</b>
Hearing loss	11	5
Hematemesis	0	13
Hematoma	0	2
Hematuria	16	7
Hemoptysis	11	0
Hepatomegaly	5	2
Hiccups	5	17
Lumbar pain	26	12
Maculopapular rash	16	14
Melena	16	8
Nausea and vomiting	68	73
Petechiae	0	8
Sore throat, odynophagia, or dysphagia	58	56
Splenomegaly	5	2
<b>Tachypnea</b>	<b>0</b>	<b>31</b>
Tinnitus	11	1



# Ebola Virus Disease (1976 and 2014)

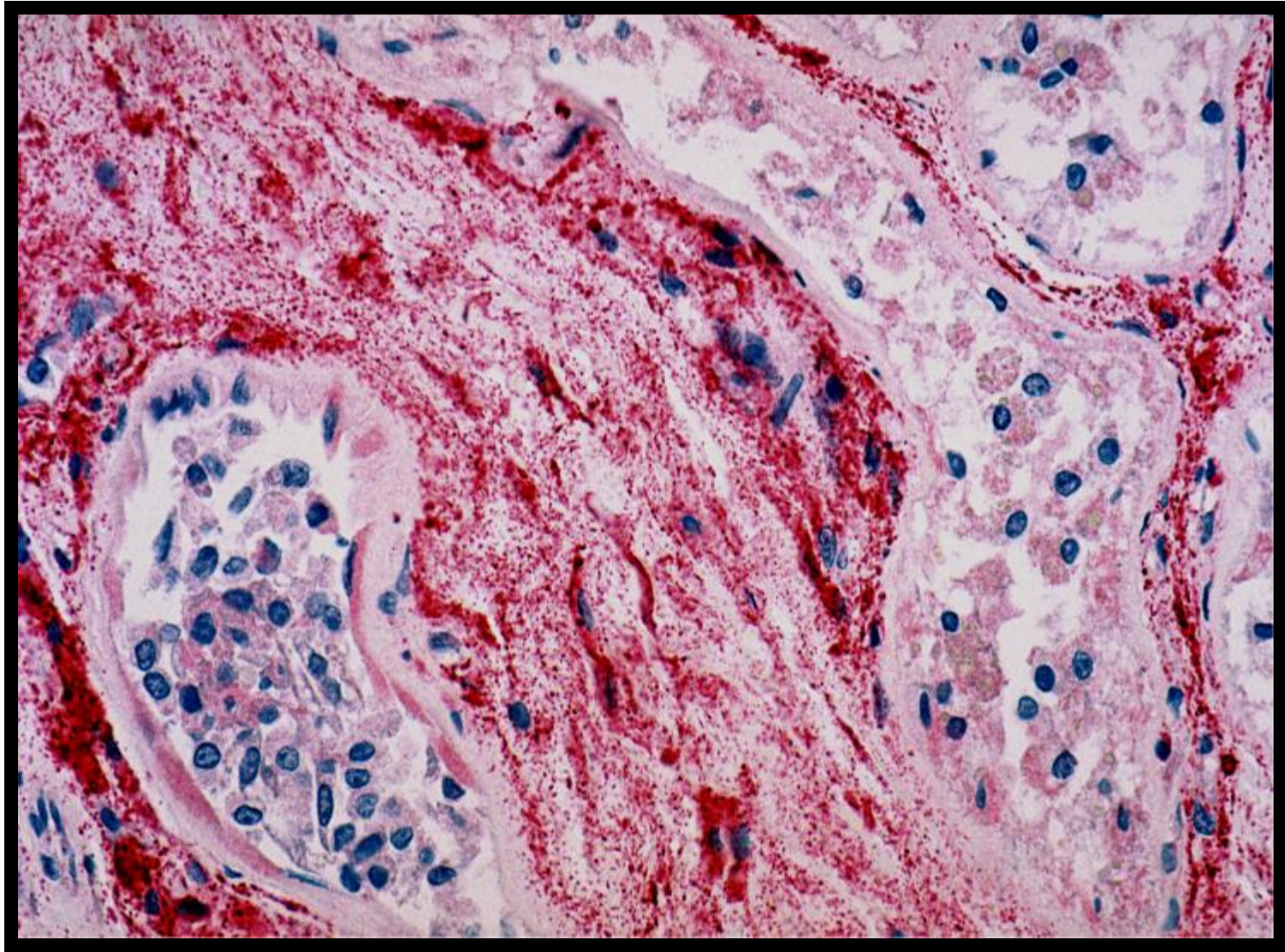


# Ebola Virus Disease, Human Liver (Zaire 1976)





## Ebola Virus Disease, Human Skin (Zaire 1976)



# Filovirus Host Search





# Filovirus Host Search



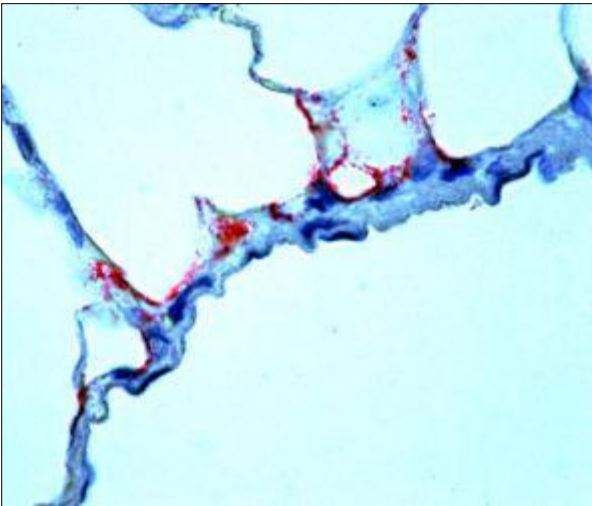
Angolan free-tailed bat (*Mops condylurus*)



Little free-tailed bat  
(*Chaerephon pumila*)



Wahlberg's epauletted fruit bat  
(*Epomophorus wahlbergi*)



# Filovirus Host Search



Social spider (*Stegodyphus dumicola*)



African brown house snake (*Lamprophis fuliginosus*)



Common house mouse (*Mus musculus*)

- *Mus setulosus*?
- *Praomys* sp.?
- *Sylvisorex ollula*?
- *Aedes Stegomyia aegypti*...?



# Filovirus Host Search



Hammer-headed fruit bat  
(*Hypsignathus monstrosus*)



Franquet's epauletted bat  
(*Epomops franqueti*)



Little-collared fruit bat  
(*Myonycteris torquata*)

# Filovirus Host Search



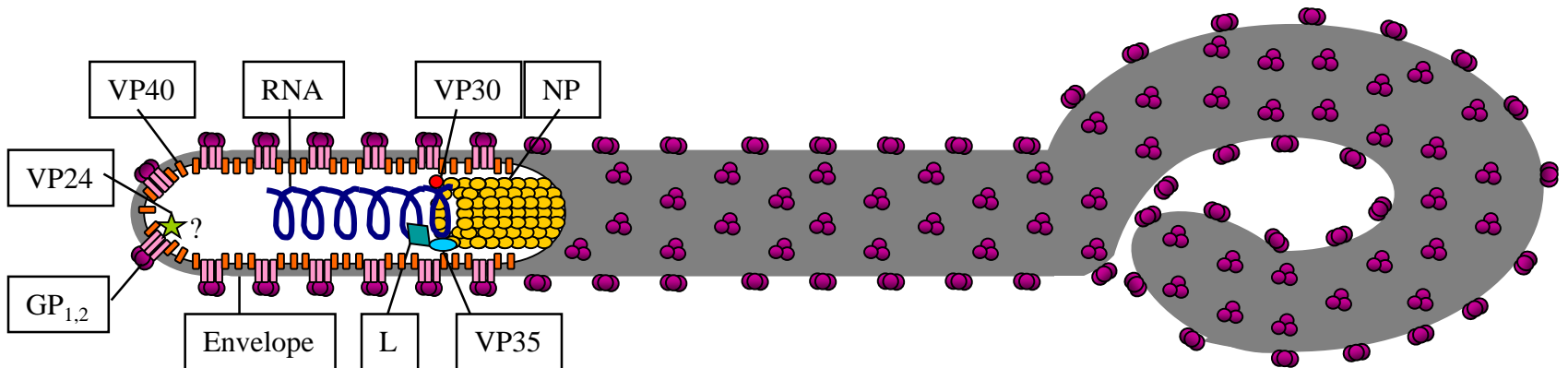
Egyptian rousette (*Rousettus aegyptiacus*)



# Filoviruses – Differences and Commonalities

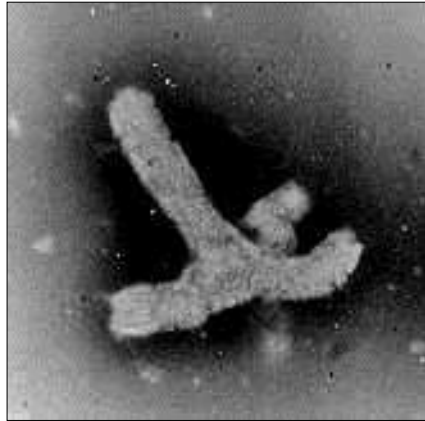
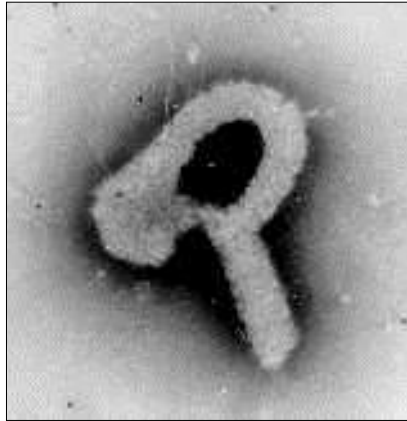
	Marburgviruses	Ebolaviruses
Antigenic cross-reactivity with members of the other genus	Minimal	Minimal
Average particle length	665 nm	805 nm
Genome length	19.1 kb	18.9 kb
Gene overlaps	One	Several
Co-transcriptional <i>GP</i> mRNA editing	No	Yes
Protein profile	Homologous sequences among all isolates, clearly distinct from ebolaviruses	Species-specific sequence differences, clearly distinct from marburgviruses
Case-fatality rate in humans	≥22-90%	≥25-90% (exceptions are Tai Forest and Reston ebolaviruses at 0%)

# Filovirus Structure



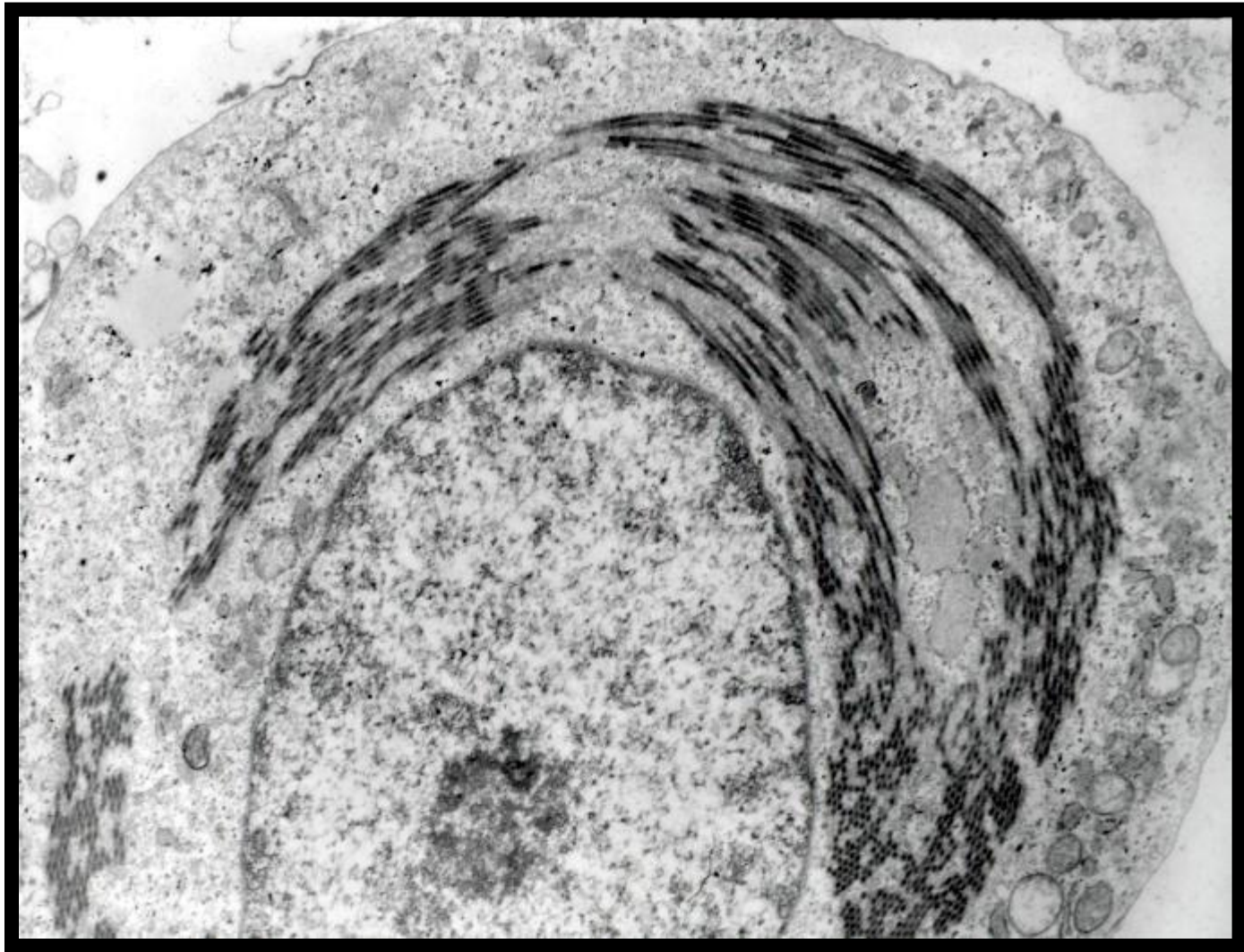
# Filovirus Morphology

(Negatively stained electron micrographs of MARV in the serum of infected guinea pigs)



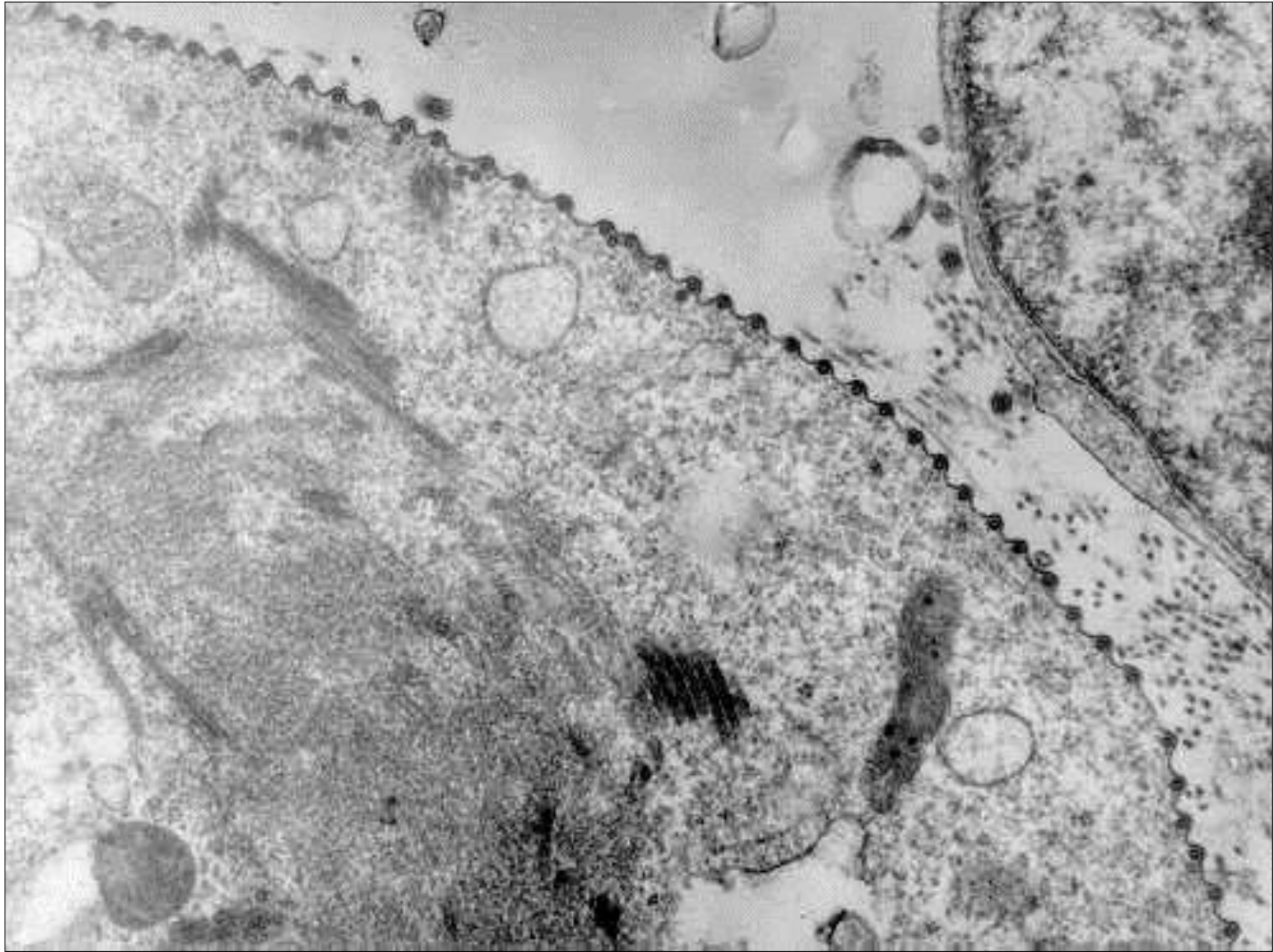
# Filovirus Morphology

(EBOV in Vero cell culture)

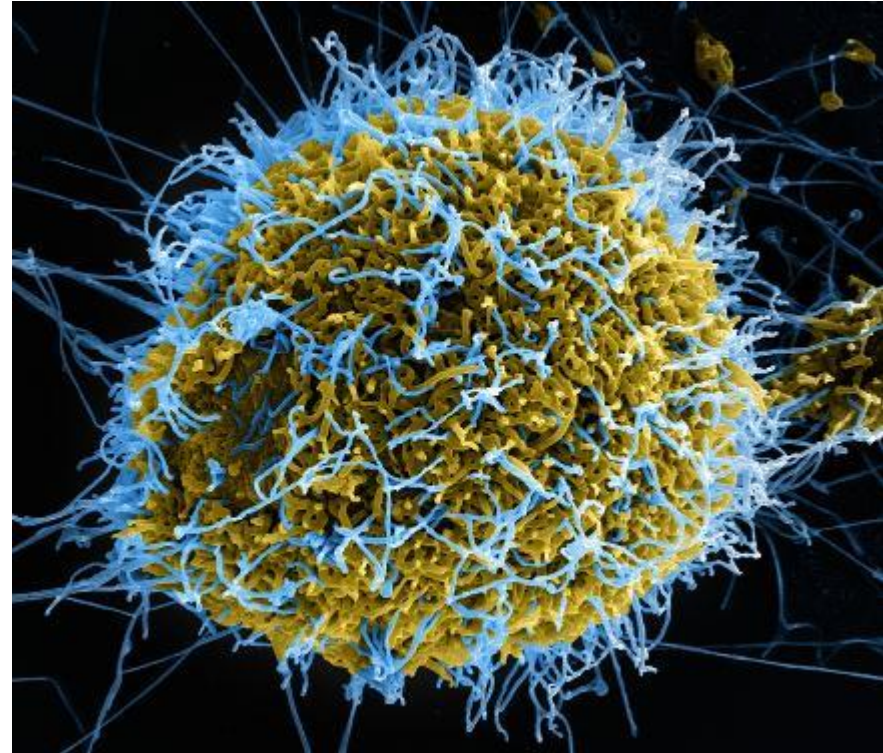
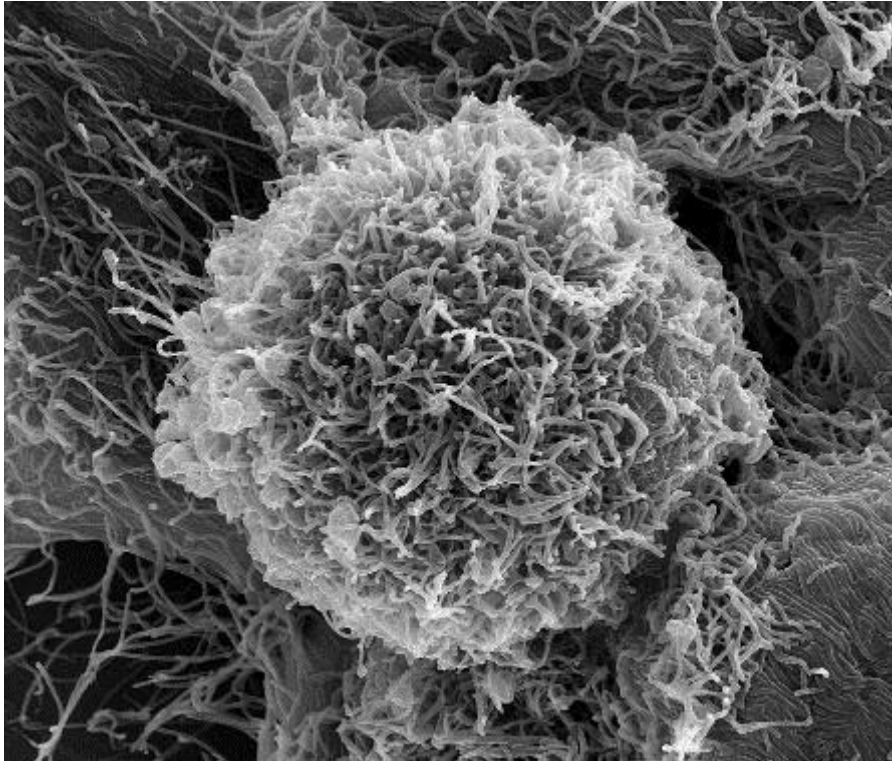




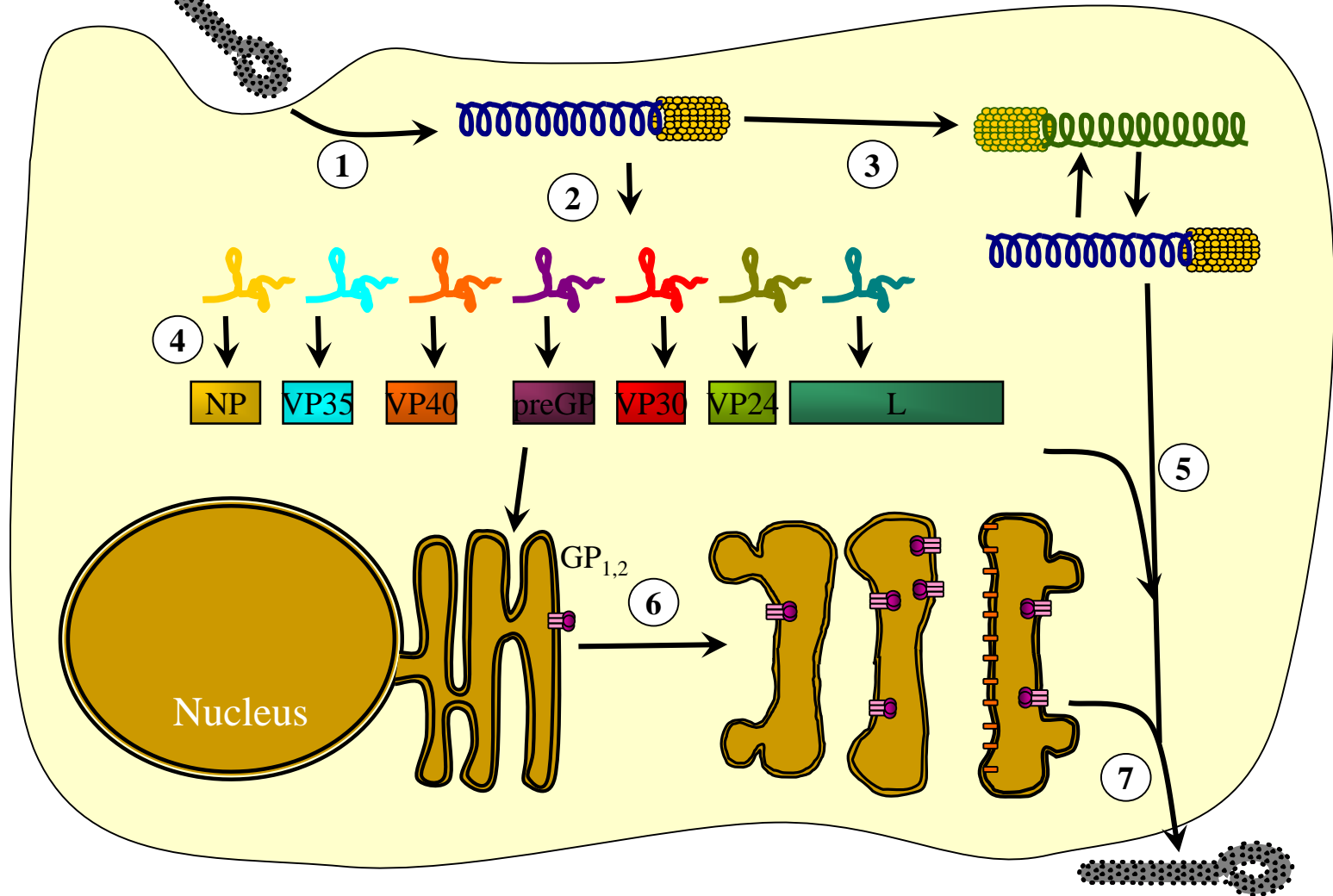
# Filovirus Morphology



# Filovirus Morphology



# Filovirus Life Cycle



# Animal Models of Filovirus Disease

Primate species subcutaneously infected with 1-10 LD <sub>50</sub> EBOV-May	Mean time to death	Hematological observations
Givets ( <i>Chlorocebus aethiops</i> )	7-8 days	Fibrin depositions
Crab-eating macaques ( <i>Macaca fascicularis</i> )	10-14 days	Microcirculatory disturbances (capillary stasis, erythrocyte aggregation), organs engorged with blood, no hemorrhage, no fibrin depositions
Hamadryas baboons ( <i>Papio hamadryas</i> )	9-10 days	Erythrocyte diapedesis
Rhesus monkeys ( <i>Macaca mulatta</i> )	7-8 days	Fibrin depositions, prominent hemorrhages



# Animal Models of Filovirus Disease

Feature of Ebola virus disease	Mice (adaptation required)	Guinea pigs (adaptation required)	Nonhuman primates	Humans
Disease duration to death	4-7 days	6-12 days	5-10 days	3-30 days
Virulence	High	High	High	High
Fever	No	Yes	Yes	Yes
Peak viremia	$\geq 7.5 \times 10^7$ - $5.6 \times 10^{11}$ pfu/ml	$\geq 10^{5.2}$ pfu/ml	$\geq 10^6$ - $10^8$ pfu/ml	$\geq 10^{6.5}$ pfu/ml
Hemorrhages	Not profound	Rare	Depending on species	Occasionally
Maculopapular rash	No	No	Depending on species	Roughly 50% of the cases
DIC	Not profound	Conflicting data	Yes	Yes
Liver enzymes	Elevated	Elevated	Elevated	Elevated
Lymphopenia	Controversial	Yes	Yes	Yes
Lymphocyte apoptosis	?	?	Yes	Yes
Thrombocytopenia	Yes	Yes	Yes	Yes
Cytokine response	Yes	Yes	Yes	Yes

# Filovirus Diagnosis

Diagnostic test	Detects	Sample Material	Advantage	Disadvantage
<b>Antigen capture ELISA</b>	Filoviral antigen	Blood, serum, tissue	Rapid, specific, sensitive	Requires special equipment (ELISA reader; $\gamma$ -irradiation of samples or handling of samples in BSL-4)
<b>Electron microscopy (EM)</b>	Complete or partial filovirus particles or characteristic inclusion bodies	Blood, serum, tissue	Specific	Insensitive, requires special equipment (electron microscope)
<b>IgG/IgM capture ELISA using native or recombinant filoviral antigen</b>	Antibodies to filoviral antigen	Serum	Rapid, specific, sensitive	Requires special equipment (ELISA reader and means to produce large amounts of purified viral or recombinant antigen)
<b>Immunohistochemistry (IHC)</b>	Filoviral antigen	Tissue (skin, liver)	Fixed tissue can be used	Requires time
<b><i>In situ</i> hybridization (ISH)</b>	Filoviral nucleic acids	Tissue	Fixed tissue can be used	Requires special equipment
<b>Indirect immunofluorescent assay (IFA) using native or recombinant filoviral antigen</b>	Antibodies to filoviral antigen	Serum	Simple, safe	Subjective interpretation, cross reactions, insensitive
<b>Reverse transcriptase-polymerase chain reaction (RT-PCR)</b>	Filoviral subgenomic or genomic nucleic acids	Blood, serum, tissue	Rapid, sensitive	Requires special equipment, possible cross-contamination (false positives), release of RT-PCR inhibitors from tissue
<b>Virus isolation in tissue culture or animals</b>	Filoviruses	Blood, tissue	Specific	Requires maximum containment laboratory and time
<b>Western blot</b>	Antibodies to filoviral antigen	Serum	Viral protein-specific	Difficult interpretation

# Prevention/Treatment/Prophylaxis

- Candidate vaccines (VSIV, adenovirus, fVLPs)
- Candidate treatments (tissue factor inhibitors, antibody cocktails, PMOs, siRNAs, small molecules)